

An intelligent collaborative system of GeneMapper® ID-X version 1.1.1. and Labvantage Sapphire™ LIMS system enables the semiautomatic data interpretation process of reference and casework samples.

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Introduction

Approximately 15,000 reference samples and 15,000 - 18,000 forensic casework samples are analyzed annually in our laboratory. Reference samples spotted on FTA® cards (Whatman) and casework samples belonging to serious crimes or property crimes are all managed in separate batches. DNA extracts from the samples are amplified with AmpFℓSTR® SGM Plus® kit (Applied Biosystems). All samples are amplified in duplicates. Amplified PCR products are run on an ABI Prism® 3130xl Genetic Analyzer and analyzed using GeneMapper® software. Before this project a modified version of GeneMapper® v4 was used in data analysis. Analyzed data are exported to the Labvantage Sapphire™ LIMS System (later LIMS system), where it is checked that the results from both PCRs are in consensus. If so, a combined final result is determined/composed, approved and sent to the DNA database (CODIS), if needed. A simplified workflow of samples is described in *Figure 1*.

Before this project, all the electropherograms of reference samples as well as casework samples were examined manually. Due to parallel analyses, this included about 65 000 electropherograms per year. About 57% of the reference samples were automatically approved using the Labvantage Sapphire™ LIMS System. The rest of the reference samples and all casework samples needed manual approval in the LIMS system. Manual result handling in GeneMapper® v4 and LIMS system was very time consuming, tedious and consumed a lot of manual human labour.

The goal of the project

The goal of the project was to reduce the turnover time and human labour needed for sample analysis by using GeneMapper® ID-X together with the LIMS as an expert system. This included verifying the GeneMapper® ID-X (v1.1.1) program to be used as an expert system with reference and property crime samples and as an expert assistant system with serious crime samples. The verification of programs for use as an expert assistant system is not described here. Artificial intelligence was built into the LIMS system to handle the data exported from GeneMapper® ID-X and to combine the results from two separate PCR reactions.

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The philosophy behind the design of the project

The design of the project was based on duplicate PCRs of all samples and on collaboration of GeneMapper® ID-X and LIMS system. GeneMapper® ID-X analysis parameters were adjusted so that it would not always be obligatory to manually examine weak, imbalanced or otherwise compromised results in the GeneMapper® ID-X program. The results from two separate PCRs from the same sample have to be in concordance with each other, but not all loci have to be determined from both PCRs.

For example if the first PCR gives the results from the first eight SGM-loci and the second PCR from the last eight SGM-loci, and the overlapping loci are in consensus, a full SGM-profile will be generated in the LIMS as a combined result. Differences in the parallel PCRs may originate for example from different PCR cycle numbers (26 and 27 cycles for reference samples) or from stochastic variation in crime scene samples with a low amount of DNA. For a reference sample to be automatically approved in the LIMS a full profile must be obtained. For casework samples six called autosomal loci is sufficient. This is the minimum loci for data base import. If both analyses represent poor data quality, the result is not automatically approved in the LIMS and the sample is examined manually. We also developed a program which saves separate PDF-plot files from every electropherogram in the GeneMapper® project. These PDF-forms of the electropherograms can be opened easily, sample by sample, using the LIMS system without opening the GeneMapper® ID-X program (details of this software are not shown here).

Furthermore, reference samples should not normally contain mixture profiles. We included a filter to the analysis parameters of the reference samples in order to cut out minor peaks and background noise. On the contrary, crime scene samples often represent mixed profiles and therefore the filter is not used in the analysis parameters of crime scene samples.

Verification of GeneMapper® ID-X (v1.1.1) program

Verification was started with allele calling concordance tests and the comparison of peak heights between the old and the new version of the program (the data is not discussed here). For several years we have gathered information about the most typical off-ladder alleles in the Finnish population. Before the implementation of GeneMapper® ID-X, the calling of these alleles was always done manually. Now, 28 of the most common off-ladder alleles were added to the GeneMapper® ID-X program to be called automatically.

Two test projects were created into the GeneMapper® ID-X (v1.1.1) program. Challenging electropherograms from reference and casework samples were pooled into these projects. Test projects included samples with low height alleles, off-scale alleles, split peaks, off ladder alleles, spikes, broad peaks, heterozygote imbalances and locus with more than two alleles. Test projects were used for the preliminary testing of different parameter sets. Reference samples and casework samples are managed in separate sample batches and they have different demands on the analysis settings. Therefore different types of parameters were chosen for different types of sample batches.

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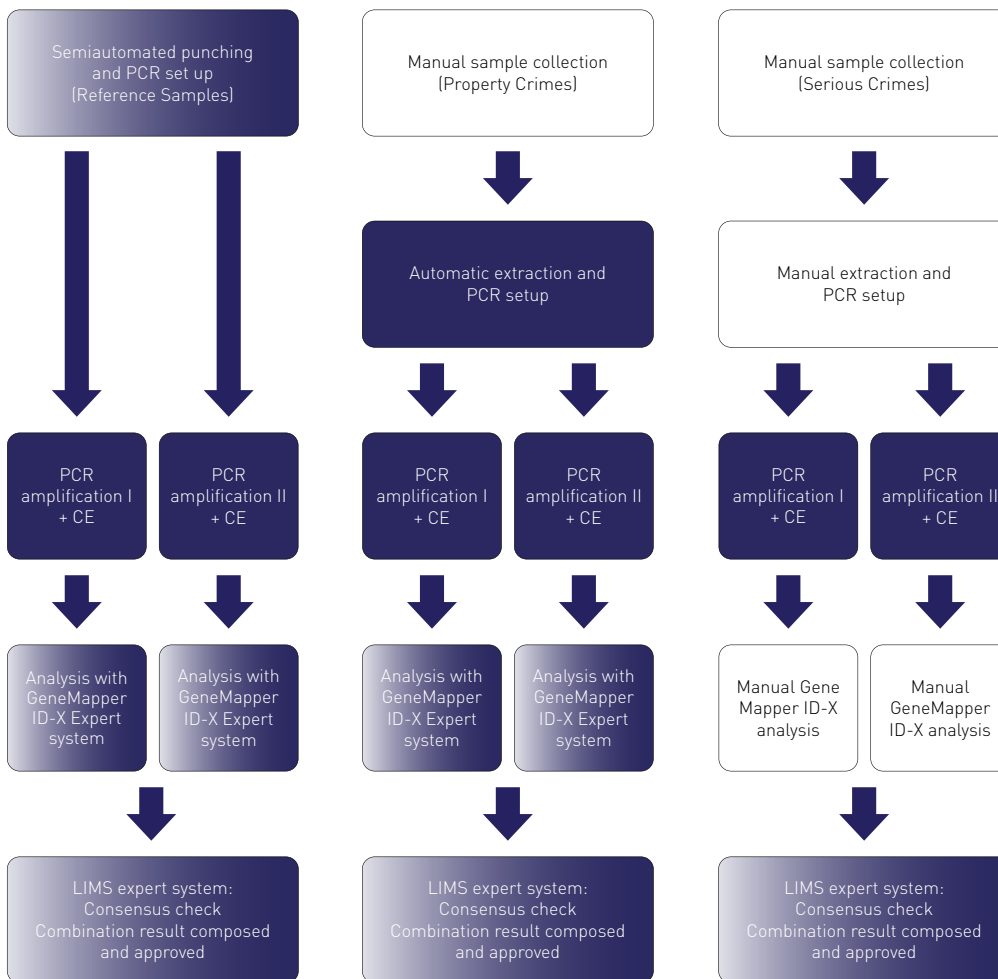


Figure 1 - Simplified workflow of reference and crime scene samples. White background indicates manual steps, shaded blue color partially automated steps and blue color fully automated steps.

Four different analysis settings were preliminary imposed and tested. The aim was to determine parameters which classifies samples according to the classes "all thresholds met" or "one or more threshold(s) not met" in the analysis summary table. The idea was that samples in the class "all thresholds met" would not later be manually examined in GeneMapper® at all. The main differences between the imposed parameters were in the peak amplitude threshold and the homozygous/heterozygous minimum peak height (data of different parameters is not shown here). Parameters were tested with the test batches (see above) and with four batches of authentic reference samples. Comparison of parameters included altogether 376 sample injections. All results were manually examined and the flag functioning was measured. The range of sample numbers falling into the category "all thresholds met" was

57 - 80%. According to the results parameters shown in *Figure 2a* were chosen. With these parameters, the number of samples falling into the category "all thresholds met" was 80% on average. Testing of these parameters was continued together with 1,678 injections. The average number of samples which passed all thresholds was around 85% (recently, around 87%, *Fig. 3*). Samples for which the category "one or more thresholds not met" was recorded in the analysis summary table showed for example off-scale alleles, low peak height alleles, heterozygote imbalances, missing size standards or more than two alleles in at least one locus. We assured the functionality of the imposed parameters over several months before finally changing the workflow accordingly.

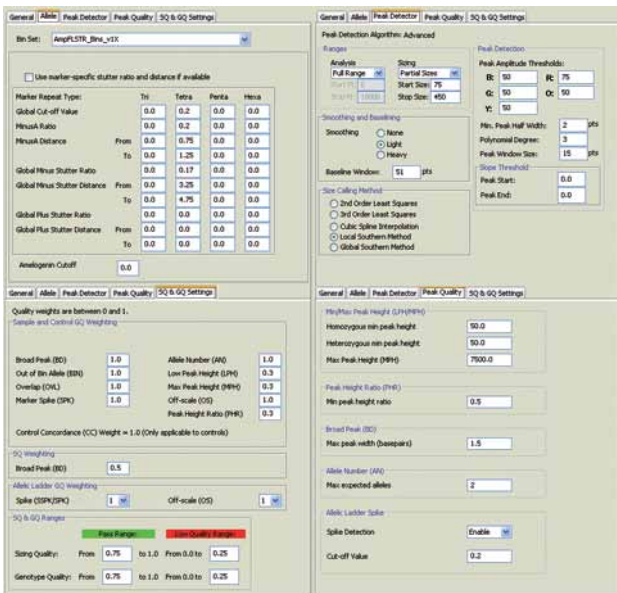


Figure 2a - Analysis parameters used for reference samples.

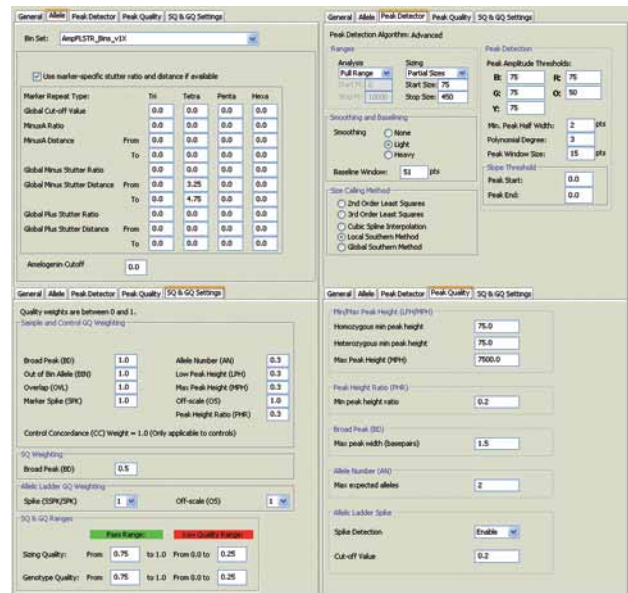


Figure 2b - Analysis parameters used for property crime samples.

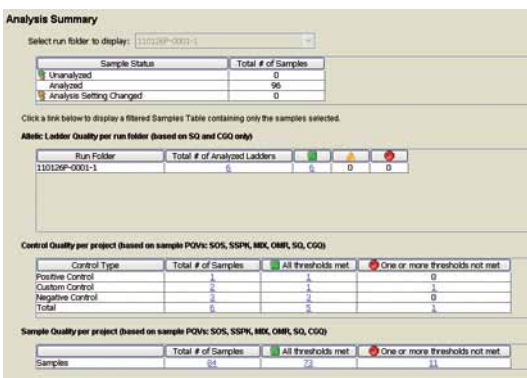


Figure 3. Analysis summary of a reference sample project in GeneMapper® ID-X. Only samples in "One or more thresholds not met" group (11 out of 84 samples) will be manually inspected.

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ITEM DESCRIPTION: K1 Base

The information received from the reference sample verification and the preliminary testing of variable parameter settings led us to test parameters presented in Figure 2b in more detail. Parameters were tested with the test batch described above and with ten batches of samples containing authentic casework samples. The overall number of injections was 710. All the electropherograms were manually examined and their categorization into classes “all thresholds met” or “one or more thresholds not met” in the analysis summary table was recorded. The average per cent of samples in the “all thresholds met” category was about 29%. The most common reason for a sample falling into category “one or more thresholds not met” was that there were no named alleles in any of the loci. These zero samples covered 30% of the samples in this category. We therefore introduced a practice into the data analysis process to arrange samples in the category “one or more analysis parameter not met” according to a “mix” column. Samples with “N/A” in this column were arranged to be at the top. Manual examination of these total zero results was skipped. This left only on average 40% of samples for manual examination. We again assured the functionality of the imposed parameters over several months before finally changing the workflow from manually examining to only manually examining samples in the category “one or more thresholds not met”.

New functions introduced into Labvantage Sapphire™ LIMS

We designed a novel automatic result approval model into the Labvantage Sapphire™ LIMS. This process compared the parallel results from duplicate PCRs locus by locus. The system takes into account concordance in allele naming, allele peak heights, number of alleles and peak height ratios. Allele peak heights were divided into classes “undersized”, “weak”, “moderate”, “strong”, “off-scale” and “imbalanced” [see *table 1*]. There are over 200 different possible combinations of parallel analyses in each locus. Accordingly, two different set of rules for result approval were set. Roughly, the first rule set measures whether the sample passes the requirement of a minimum amount of similarity between parallel analyses (consensus between the results). The second rule set measures locus by locus whether a given result combination passes the requirements of automatic approval. The second rule set is used only if a sample passed the first set of rules. Rules for reference samples are slightly different than rules for casework samples (details of rules are not discussed here). All these rules are stricter than the rules defined for manual approval where every EPG is inspected by the human eye. Only the essential features of this multiform system are described here.

Automatic result approval of the system starts when a combined table of a sample batch is exported from GeneMapper® ID-X into LIMS. Before export, the LIMS checks if all positive and negative controls in the sample batch have passed. If not, the automatic approval system is suspended and all the results have to be approved manually. This is a function that one would like to use for example if there is a contamination in one of the controls. If all the controls have passed, the following chain of actions will start:

1. During the first step, the results of the duplicate PCRs are compared locus by locus following strict rules (consensus check). If the result pair is classified as “accept” (strong results from both PCRs, in concordance with each other), the alleles of the locus are copied to the “combination result” cell. If less than six autosomal loci (crime scene samples) or five autosomal loci (FTA-samples) fulfil the “accept” category, the process is suspended and the sample has to be examined manually. If the consensus check passes, the process moves to the next step.

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2. During the second step, all results are compared again locus by locus according to less strict rules. For example if a locus fails in the first PCR but gives a strong result in the second PCR, the second result is copied to the “combination result” cell. If there is any incongruity between the results or the result pair is classified as “do not accept”, the “combination result” cell is left empty. For casework samples it is also possible to fill the “combination result” cell for a certain locus with value “0”, giving partial DNA profiles. After checking all the loci of the sample, if all “combination result” cells are filled, the result is locked, approved and sent to the DNA database, if needed. Also, the frequency of the profile in the Finnish population is calculated automatically. If any of the “combination result” cells are not filled, the sample is transferred for manual inspection.

If the sample has been examined manually in the GeneMapper® ID-X and the user has added one of the predefined marks into the sample’s UD1 column, the previously described process is suspended. The UD1 column is used for example if an off-ladder allele has been manually called or if there are doubts about contamination.

The new functions described above were first introduced into the test environment of the LIMS system. For functionality testing, manipulated combined tables of the GeneMapper® ID-X were produced. All result combinations needed were included into these tables. Testing was further extended to authentic reference samples (1,092 pieces), property crime scene samples (605 pieces) and serious crime scene samples (383 pieces). After testing, the new functions were consigned into the production environment. The average proportion of automatically accepted samples was 81% (reference samples), 53% (property crime casework samples) and 33% (serious crime casework samples). Information transported from the GeneMapper® ID-X into the LIMS system is listed in *Table 1*. Part of the information used by automatic acceptance system and another part supplements the manual examination process.

Table 1 - Information transported from GeneMapper® ID-X into Labvantage Sapphire™ LIMS System (simplified)

Data in GeneMapper ID-X combined table	Usage of data
Allelic peak heights:	
Homozygote locus 50-74 RFU*	“under sizes” (supplementary information)
Homozygote locus 75-149 RFU	“weak” locus
Homozygote locus 150-299 RFU	“moderate” locus
Homozygote locus ≥300 RFU	“strong” locus
Homozygote locus >7500 RFU	“off-scale” locus
Heterozygote locus 50-74 RFU*	“under sized” (supplementary information)
Heterozygote locus 75-149 RFU	“moderate” locus
Heterozygote locus ≥150 RFU	“strong” locus
Heterozygote locus >7500 RFU	“off-scale” locus
Imbalance in alleles in heterozygote locus (>50%)	“imbalanced locus”
SOS flag information	supplementary information
SQ flag information	supplementary information
SSPK flag information	supplementary information
OMR flag information	supplementary information
Comment in UD1 column	hinders automatic approval
Comment in UD2 column	supplementary information
Comment in UD3 column	supplementary information

* These alleles are labelled only in reference samples

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Summary

After this project reference samples as well as property casework samples fall into 3 different interpretation categories:

1. Interpretation process is fully automatic. Samples do not need manual examination in the GeneMapper® ID-X and the results are automatically accepted in LIMS system.
2. Interpretation is partially automatic. Samples need manual examination in the GeneMapper® ID-X, but the results are automatically accepted in LIMS-system or vice a versa.
3. Interpretation is fully manual. Samples need manual examination both in the GeneMapper® ID-X and in LIMS system (manual acceptance or re-run).

The outcome of the project is that about 87% of reference samples do not need manual examination in the GeneMapper® ID-X (in GeneMapper® v4 every sample was manually examined). 81% of reference samples are automatically accepted into the LIMS system (before this project this was 57%). In property crime scene sample batches, corresponding portions are 60% and 53%, respectively. For serious casework samples, it was decided to not use GeneMapper® ID-X as an expert system at this point. This will however be considered in future. Automatic LIMS acceptance system was nonetheless also extended to serious casework samples. About 33% of samples are automatically accepted. Different sample types have different GeneMapper® ID-X analysis parameters as well as different established practices in the manual examination of electropherograms. These are the main reasons for the different percentages in automatically approved sample numbers in the LIMS system.

In conclusion, we have designed this project as an intelligent collaborative system of the GeneMapper® ID-X and the Labvantage Sapphire™ software for the semi-automatic data interpretation process. The system has proven to work reliably. We have estimated that the partially automated analysis of samples saves one year in staff resources. The turnaround time of sample analysis is also reduced. We are probably one of the first laboratories to have included automation to result interpretation of casework samples. This nicely completes the validated automatic analysis line (DNA extraction and PCR set up) of property crime scene samples. The described design is made for AmpFℓSTR® SGM Plus® kit (Applied Biosystems) amplification results. The system is however flexible and can easily be modified when new amplification kits are adopted.

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